L20 ANSWER 12 OF 15 PCTFULL COPYRIGHT 2003 Univentio
ACCESSION NUMBER: 1995029239 PCTFULL ED 20020514
TITLE (ENGLISH): COMPOUNDS AND METHODS FOR THE STIMULATION AND ENHANCEMENT OF PROTECTIVE IMMUNE RESPONSES AND **IL-12** PRODUCTION
TITLE (FRENCH): COMPOSES ET METHODES PERMETTANT DE STIMULER ET DE FAVORISER LES REPONSES IMMUNITAIRES PROTECTRICES ET LA PRODUCTION DE **IL-12**
INVENTOR(S): REED, Steven, G.
PATENT ASSIGNEE(S): CORIXA CORPORATION
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9529239	**A2**	**19951102**

DESIGNATED STATES
W: AM AU BB BG BR BY CA CN CZ EE ES FI GE HU IS JP KE KG KP KR KZ LK LR LT LV MD MG MN MW MX NO NZ PL RO RU SD SG SI SK TJ TT UA UG UZ VN KE MW SD SZ UG AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
APPLICATION INFO.: WO 1995-US5064 A 19950424
PRIORITY INFO.: US 1994-8/232,534 19940422

ABEN Compounds and methods for stimulating and enhancing immune responses and
for evaluating patient
immune responses are disclosed. Disclosed compounds include polypeptides
that contain at least a
biologically active portion of a Leshmania braziliensis homolog of the eukaryotic initiation factor
4A, or a variant thereof. Such compounds are useful for stimulating a Th1 immune response and **IL-12**
production in patients, as well as in isolated cells and cell cultures. The polypeptides of this
invention are further useful for the evaluation and treatment of patients, who may be afflicted with
leishmaniasis or other disorders.

ABFR Composes et methodes destines a stimuler et a ameliorer les reponses immunitaires et a evaluer
les reponses immunitaires de patients. Les composes decrits comprennent des polypeptides qui
contiennent au moins une partie biologiquement active d'un homologue Leishmania braziliensis du
facteur d'initiation eucaryote 4A, ou une variante de ladite partie. Lesdits composes sont utiles
pour stimuler une reponse immunitaire Th1 et la production d'interleukine-12 (**IL-12**) chez des
patients, ainsi que dans des cellules isolees et des cultures cellulaires. Les polypeptides de la
presente invention sont en outre utiles pour evaluer et traiter des patients qui peuvent etre
atteints de leishmaniose et d'autres troubles.

L20 ANSWER 5 OF 15 PCTFULL COPYRIGHT 2003 Univentio
ACCESSION NUMBER: 1997000321 PCTFULL ED 20020514
TITLE (ENGLISH): IMMUNE RESPONSE MODULATORS AND USES THEREFOR
TITLE (FRENCH): MODULATEURS DE LA REPONSE IMMUNITAIRE ET LEUR UTILISATION
INVENTOR(S): SEOW, Heng-Fong;
WOOD, Paul
PATENT ASSIGNEE(S): COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION;
SEOW, Heng-Fong;
WOOD, Paul
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9700321	**A1**	**19970103**

DESIGNATED STATES
W: AL AM AT AU AZ BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU IL IS JP KE KG KP KR KZ LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG US UZ VN KE LS MW SD SZ UG AM AZ BY KG KZ MD RU TJ TM AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG

APPLICATION INFO.:	WO 1996-AU360	A	19960614
PRIORITY INFO.:	AU 1995-PN 3502		19950614
	AU 1995-PN 6244		19951027

ABEN The present invention relates to a nucleic acid molecule comprising a nucleotide sequence encoding, or complementary to a sequence encoding, an ovine IL-5 or **IL-12** cytokine molecule. The invention further provides recombinant isolated ovine IL-5 and **IL-12** polypeptides which are useful as immune response modulators in livestock animals.

ABFR La presente invention concerne une molecule d'acide nucleique qui comprend une sequence de nucleotides codant une molecule de cytokine IL-5 ou **IL-12** d'ovins ou qui est complementaire d'une telle sequence codant une telle molecule de cytokine. L'invention traite aussi de polypeptides IL-5 et **IL-12** isoles et de recombinaison, d'ovins, qui peuvent etre utilises comme modulateurs de la reponse immunitaire pour le betail.

L20 ANSWER 11 OF 15 PCTFULL COPYRIGHT 2003 Univentio
ACCESSION NUMBER: 1996001272 PCTFULL ED 20020514
TITLE (ENGLISH): HELICOBACTER PROTEINS AND VACCINES
TITLE (FRENCH): PROTEINES D'HELICOBACTER ET VACCINS
INVENTOR(S): KELLEHER, Dermot;
WINDLE, Henry;
BYRNE, William;
McMANUS, Ross
PATENT ASSIGNEE(S): RICAN LIMITED;
KELLEHER, Dermot;
WINDLE, Henry;
BYRNE, William;
McMANUS, Ross
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9601272	**A1**	**19960118**

DESIGNATED STATES
W: AM AT AU BB BG BR BY CA CH CN CZ DE DE DK DK EE ES FI GB GE HU IS JP KE KG KP KR KZ LK LR LT LU LV MD MG MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TT UA UG US UZ VN KE MW SD SZ UG AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG

APPLICATION INFO.:	WO 1995-IE36	A	19950703
PRIORITY INFO.:	IE 1994-940538		19940701
	IE 1995-950249		19950406

ABEN A vaccine includes at least one Helicobacter, especially Helicobacter pylori protein to which immunoreactivity is detected in H. pylori negative individuals. The Helicobacter proteins are preferably less than 30 kDa and the vaccine especially includes 24 to 25 kDa and/or 18 to 19 kDa proteins. The vaccine may include **interleukin (12)** as an **adjuvant**.

ABFR La presente invention concerne au moins une proteine d'Helicobacter, en l'occurrence une proteine d'Helicobacter pylori contre laquelle une immunoreactivite a ete detectee chez des individus H.Pylori-negatifs. Les proteines d'Helicobacter sont de preference inferieures a 30 kDa, et le vaccin comprend des proteines de 24 a 25 kDa et/ou de 18 a 19 kDa. Le vaccin peut comporter de l'interleukine 12 comme **adjuvant**.

L20 ANSWER 9 OF 15 PCTFULL COPYRIGHT 2003 Univentio
ACCESSION NUMBER: 1996011019 PCTFULL ED 20020514
TITLE (ENGLISH): **INTERLEUKIN-12** AS AN **ADJUVANT** FOR PARAMYXOVIRIDAE VACCINES
TITLE (FRENCH): INTERLEUKINE-12 UTILISEE EN TANT QU'**ADJUVANT** POUR VACCINS CONTRE LES PARAMYXOVIRIDAE
INVENTOR(S): GRAHAM, Barney, S.;
TANG, Yi-Wei
PATENT ASSIGNEE(S): VANDERBILT UNIVERSITY
LANGUAGE OF PUBL.: English
DOCUMENT TYPE: Patent
PATENT INFORMATION:

NUMBER	KIND	DATE
WO 9611019	**A1**	**19960418**

DESIGNATED STATES
W: AU CA JP MX AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
APPLICATION INFO.: WO 1995-US12656 A 19951002
PRIORITY INFO.: US 1994-8/318,480 19941005

ABEN A method is disclosed of reducing viral replication of a virus of the Paramyxoviridae family in a host, comprising administering to the host an antigen of the virus in combination whith an effective **adjuvant** amount of **interleukin-12** (**IL-12**). Human viruses of the Paramyxoviridae family include paramyxoviruses (e.g., parainfluenza virus 1, parainfluenza virus 2, parainfluenza virus 3 and parainfluenza virus 4), morbilliviruses (e.g., measles virus) and pneumoviruses (e.g., respiratory syncytial virus); other non-human viruses of the Paramyxoviridae family include canine distemper virus, bovine respiratory syncytial virus, Newcastle disease virus and rhinderpest virus. A composition is also disclosed comprising a mixture of an antigen of a virus of the Paramyxoviridae family and an effective **adjuvant** amount of **interleukin -12** (**IL-12**).

ABFR L'invention concerne un procede de reduction de la replication virale d'un virus de la famille des paramyxoviridae chez un hote, consistant a administrer a ce dernier un antigene du virus en combinaison avec une dose **adjuvante** efficace d'interleukine-12 (**IL-12**). Les virus humains de la famille des paramyxoviridae comprennent les paramyxovirus (par exemple le virus parainfluenza 1, le virus parainfluenza 2, le virus parainfluenza 3 et le virus parainfluenza 4), les morbillivirus (par exemple, le virus morbilleux) et les pneumovirus (par exemple, le virus respiratoire syncytial); d'autres virus non humains de la famille des paramyxoviridae comprennent le virus de la maladie de Carre, le virus respiratoire syncytial bovin, le virus de la maladie de Newcastle, et le virus de la peste bovine. L'invention concerne egalement une composition comprenant un melange d'un antigene

d'un virus de la famille des paramyxoviridae ainsi qu'une dose **adjuvante** efficace d'interleukine-12 (**IL-12**).

L16 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1995:501322 CAPLUS
DOCUMENT NUMBER: 122:237772
TITLE: Low-molecular-weight proteins released by mycobacteria, their manufacture with recombinant cells, and their use in diagnosis and in **tuberculosis** vaccines
INVENTOR(S): Andersen, Peter; Andersen, Aase Bengaard; Hasloev, Kaare; Soerensen, Anne Lund
PATENT ASSIGNEE(S): Statens Serumsinstitut, Den.
SOURCE: PCT Int. Appl., 100 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
WO 9501441	A1	19950112	WO 1994-DK273	19940701	<--
W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, CZ, DE, DE, DK, DK, ES, FI, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, SK, TJ, TT, UA					
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG					
CA 2165949	AA	19950112	CA 1994-2165949	19940701	<--
AU 9470688	A1	19950124	AU 1994-70688	19940701	<--
AU 682879	B2	19971023			
EP 706571	A1	19960417	EP 1994-919574	19940701	<--
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE					
US 5955077	A	19990921	US 1995-465640	19950605	

PRIORITY APPLN. INFO.:
DK 1993-798 19930702
US 1993-123182 19930920
WO 1994-DK273 19940701

AB The invention relates to a secreted antigenes from mycobacteria capable of evoking early (within 4 days) immunol. responses from T-helper cells in the form of .gamma.-interferon release in memory immune animals after rechallenge infection with mycobacteria of the **tuberculosis** complex. The antigens are present in short term culture filtrates from cultured mycobacteria belonging to the **tuberculosis** complex. One of these antigens, a polypeptide with an apparent mol. wt. of 6 kDa, has been identified, and the DNA encoding the polypeptide has been cloned and sequenced. Also disclosed are methods of immunizing animals/humans and methods of diagnosing **tuberculosis**.

L5 ANSWER 5 OF 6 DGENE (C) 2003 THOMSON DERWENT
ACCESSION NUMBER: AAT71599 DNA DGENE
TITLE: Vaccines derived from M.tuberculosis major abundant extracellular proteins - are easy to prepare and less toxic than conventional killed or attenuated vaccines, useful for protecting against or treating Mycobacterial infections
INVENTOR: Harth G; Horwitz M A
PATENT ASSIGNEE: (REGC)UNIV CALIFORNIA.
PATENT INFO: WO 9637219 A1 19961128 193p
APPLICATION INFO: WO 1996-US7781 19960523
PRIORITY INFO: US 1995-568357 19951206
US 1995-447398 19950523
US 1995-545926 19951020
US 1995-551149 19951031
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 1997-020936 [02]
CROSS REFERENCES: P-PSDB: AAW18165
DESCRIPTION: Mycobacterium tuberculosis extracellular 32AKD protein encoding DNA.

AN AAT71599 DNA DGENE
NA 164 A; 333 C; 349 G; 171 T; 0 other
SQL 1017
SEQ

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HITS AT: 501-530

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<212> DNA
<213> Mycobacterium tuberculosis

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L25 ANSWER 6 OF 10 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 95248852 MEDLINE
DOCUMENT NUMBER: 95248852 PubMed ID: 7537350
TITLE: Sustained T-cell reactivity to Mycobacterium tuberculosis specific antigens in 'split-anergic' leprosy.
AUTHOR: Kaleab B; Wondimu A; Likassa R; Woldehawariat N; Ivanyi J
CORPORATE SOURCE: MRC Clinical Sciences Centre, Royal Postgraduate Medical School, Hammersmith Hospital, London, UK.
SOURCE: LEPROSY REVIEW, (1995 Mar) 66 (1) 19-25.
Journal code: 0243711. ISSN: 0305-7518.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199506
ENTRY DATE: Entered STN: 19950608
Last Updated on STN: 19960129
Entered Medline: 19950601

AB Split anergy represented by delayed-type hypersensitivity skin reaction to tuberculin, but not to leprosin, is known to occur in a distinct proportion of leprosy patients. The mechanism was originally attributed to Mycobacterium leprae-specific suppression of T cells toward common mycobacterial antigens. This study ascertained an alternative explanation, attributing the phenomenon to selective responsiveness to M. tuberculosis-specific epitopes. Indeed, the results of blood T-cell proliferative responses in 11 split-anergic patients showed normal responsiveness to the M. tuberculosis-specific 38 kDa lipoprotein and peptide 71-91 of the **16** kDa antigen but diminished responsiveness to 2 common mycobacterial antigens, represented by the 65 kDa heat shock protein and the fibronectin-binding **Ag85** complex, as compared with leprosin responsive patients and healthy contacts. These findings support the hypothesis that split anergy is due to selective recognition of M. tuberculosis-specific epitopes and deletion of T cells reacting to shared mycobacterial antigens.

lungs from B6 mice, subsequently challenged. Therefore, this technique may be useful for the definition of protective antigens of M. **tuberculosis** and the development of a more effective **tuberculosis** vaccine.

L11 ANSWER 3 OF 27 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 1998164681 MEDLINE
DOCUMENT NUMBER: 98164681 PubMed ID: 9503930
TITLE: [Examining of humoral immunity on mycobacteria antigens in sarcoidosis].
Izuchenie gumoral'nogo immuniteta na antigeny mikobakterii pri sarkoidoze.
AUTHOR: Litvinov V I; Chernousova L N; Kalinina O A; Safonova S G; Dem'ianenko N V; Kulikovskaia N V; Kapina M A
SOURCE: PROBLEMY TUBERKULEZA, **(1997)** (6) 40-2.
Journal code: PQE; 0414141. ISSN: 0032-9533.
PUB. COUNTRY: RUSSIA: Russian Federation
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Russian
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199804
ENTRY DATE: Entered STN: 19980422
Last Updated on STN: 19980422
Entered Medline: 19980414

AB Antibodies to Mycobacterium **tuberculosis** antigens H37Rv and reverse strains previously isolated from patients with sarcoidosis with granular isolates were determined in 50 patients with sarcoidosis (including 16 patients isolating granular types) and 56 patients with **tuberculosis**, by using ELISA and immunoblotting. Serum antibodies from patients with sarcoidosis were ascertained to more commonly react in ELISA with the antigen (ultrasound disintegrant (USDs) obtained from reverse mycobacteria isolated (initially) from patients with sarcoidosis (AGS) than with the USD of the M. **tuberculosis** H37Rv (AGT) and, on the contrary, serum antibodies from patients with **tuberculosis** more frequently reacted with the M. **tuberculosis** H37Rv. The spectrum of serum antibodies from patients with sarcoidosis greatly differed at immunoblotting with AGS and AGT. There was most commonly a reaction with the antigenic determinants 79, 27, 30, and 50 kDa to AGS and that of the determinants 17, 35, **32 kDa** to AGT.

L11 ANSWER 4 OF 27 BIOTECHDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1997-01373 BIOTECHDS
TITLE: Vaccines against Mycobacterium infection containing major abundant extracellular proteins;
Mycobacterium tuberculosis recombinant protein preparation
by vector expression in Escherichia coli, Mycobacterium smegmatis or Mycobacterium vaccae for use as a recombinant vaccine
AUTHOR: Horwitz M A; Harth G
PATENT ASSIGNEE: Univ.California
LOCATION: Oakland, CA, USA.
PATENT INFO: WO 9637219 **28 Nov 1996**
APPLICATION INFO: WO 1996-US7781 23 May 1996
PRIORITY INFO: US 1995-568357 6 Dec 1995; US 1995-447398 23 May 1995
DOCUMENT TYPE: Patent
LANGUAGE: English

OTHER SOURCE: WPI: 1997-020936 [02]
AN 1997-01373 BIOTECHDS
AB A vaccinating agent for use in promoting an effective immune response in a mammalian host against an infectious pathogen from the genus Mycobacterium is claimed, which consists of at least a portion of at least one mainly abundant extracellular protein (Mycobacterium **tuberculosis** 110 kD, 80 kD, 71 kD, 58 kD, 45 kD, **32A kD**, 32B kD, 30 kD, 24 kD, 23.5 kD, 23 kD, 16 kD, 14 kD, 12 kD proteins and their respective analogs, homologs and subunits thereof). Also claimed are: (a) DNA (sequences specified) encoding the M. **tuberculosis** proteins, optionally operably linked to a eukaryotic promoter sequence; (b) protein sequences (specified) encoded by the DNA; (c) vaccinating agents containing DNA able to express the proteins when introduced in vivo; (d) vaccinating agents containing at least one immunodominant epitope of the protein; (e) immunodiagnostic agents containing at least one of these epitopes; and (f) vector plasmid pET22b and plasmid pSMT3 containing the DNA for use in the recombinant production of the proteins by gene expression in a host cell, preferably Escherichia coli, Mycobacterium smegmatis or Mycobacterium vaccae. (192pp)

L11 ANSWER 5 OF 27 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 96333337 MEDLINE
DOCUMENT NUMBER: 96333337 PubMed ID: 8757831
TITLE: Novel insights into the genetics, biochemistry, and immunocytochemistry of the 30-kilodalton major extracellular protein of Mycobacterium tuberculosis.
COMMENT: Erratum in: Infect Immun 1997 Feb;65(2):852
AUTHOR: Harth G; Lee B Y; Wang J; Clemens D L; Horwitz M A
CORPORATE SOURCE: Division of Infectious Diseases, Department of Medicine, University of California Los Angeles School of Medicine 90095, USA.
CONTRACT NUMBER: AI-07126 (NIAID)
AI-33138 (NIAID)
AI-35275 (NIAID)
SOURCE: INFECTION AND IMMUNITY, **(1996 Aug)** 64 (8) 3038-47.
Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U38939; GENBANK-U47335
ENTRY MONTH: 199609
ENTRY DATE: Entered STN: 19961008
Last Updated on STN: 19980206
Entered Medline: 19960926
AB The 30/**32-kDa** complex of major secretory proteins are among the most important and intensively studied proteins of Mycobacterium **tuberculosis**. The proteins have been demonstrated to be immunoprotective and to play a central role in the physiology of the mycobacterium. In this study, we present a series of novel insights into this key protein complex arising out of a combination of genetic, biochemical, and immunocytochemical analyses. Our genetic analyses (i) indicate that the genes are arranged as separate transcription units, (ii) demonstrate that the mature 30-kDa protein of M. **tuberculosis** differs from the corresponding 30-kDa proteins of two strains of Mycobacterium bovis BCG by only 1 and 5 amino acids, (iii) suggest that

expression of the proteins is regulated at the transcriptional level, and (iv) map the transcriptional start site of the 30-kDa protein gene. Our biochemical analyses provide evidence that (i) the 30-kDa protein and the two **32-kDa** proteins (i.e., 32A and 32B) are secreted at a ratio of approximately 3:2:1, respectively, (ii) the proteins exist as monomers, (iii) the proteins are not posttranslationally modified by the addition of carbohydrates and lipids, (iv) the 30-kDa and 32A proteins contain one disulfide bridge, and (v) high-level expression and leader peptide processing are achievable in Escherichia coli. Our immunocytochemical analyses demonstrate that the 30/**32-kDa** complex is expressed in human monocytes and that the proteins are localized to the phagosomal space and the mycobacterial cell wall. These analyses fill important gaps in our knowledge of this critical protein complex of M. **tuberculosis** and, at the same time, raise new and fundamental questions regarding regulatory mechanisms that control coordinate expression of the proteins at a fixed ratio.

L11 ANSWER 6 OF 27 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 96379040 MEDLINE
DOCUMENT NUMBER: 96379040 PubMed ID: 8784603
TITLE: Comparison of amplicor and 32-kilodalton PCR for detection of Mycobacterium tuberculosis from sputum specimens.
AUTHOR: Soini H; Agha S A; El-Fiky A; Viljanen M K
CORPORATE SOURCE: Department in Turku, National Public Health Institute, Finland.
SOURCE: JOURNAL OF CLINICAL MICROBIOLOGY, **(1996 Jul)** 34 (7) 1829-30.
Journal code: HSH; 7505564. ISSN: 0095-1137.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199612
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19961209
AB The commercial PCR test Amplicor was compared with the **32-kDa** PCR for detection of Mycobacterium **tuberculosis** from 76 sputum specimens from Egyptian patients. Both tests performed with rather equal efficacy (resolved sensitivity of 88.9% for both tests; specificity of 98.0% for Amplicor and 93.9% for **32-kDa** PCR). PCR was found to be useful in detection of auramine fluorescent stain-positive, culture-negative specimens.

L11 ANSWER 7 OF 27 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 96380995 MEDLINE
DOCUMENT NUMBER: 96380995 PubMed ID: 8789008
TITLE: Multiprimer PCR system for differential identification of mycobacteria in clinical samples.
AUTHOR: Del Portillo P; Thomas M C; Martinez E; Maranon C; Valladares B; Patarroyo M E; Carlos Lopez M
CORPORATE SOURCE: Departamento de Biologia Molecular, Instituto de Parasitologia y Biomedicina, Granada.
SOURCE: JOURNAL OF CLINICAL MICROBIOLOGY, **(1996 Feb)** 34 (2) 324-8.
Journal code: HSH; 7505564. ISSN: 0095-1137.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199610
ENTRY DATE: Entered STN: 19961106
Last Updated on STN: 19990129
Entered Medline: 19961021

AB A novel multiprimer PCR method with the potential to identify mycobacteria in clinical samples is presented. The assay relies on the simultaneous amplification of three bacterial DNA genomic fragments by using different sets of oligonucleotide primers. The first set of primers amplifies a 506-bp fragment from the gene for the **32-kDa** antigen of Mycobacterium **tuberculosis**, which is present in most of the species belonging to the genus Mycobacterium. The second set of primers amplifies a 984-bp fragment from the IS6110 insertion sequence of the bacteria belonging to the M. **tuberculosis** complex. The third set of primers, derived from an M. **tuberculosis** species-specific sequence named MTP40, amplifies a 396-bp genomic fragment. Thus, while the multiprimer system would render three amplification fragments from the M. **tuberculosis** genome and two fragments from the Mycobacterium bovis genome, a unique amplification fragment would be obtained from nontuberculous mycobacteria. The results obtained, using reference mycobacterial strains and typed clinical isolates, show that the multiprimer PCR method may be a rapid, sensitive, and specific tool for the differential identification of various mycobacterial strains in a single-step assay.

L11 ANSWER 8 OF 27 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 96352985 MEDLINE
DOCUMENT NUMBER: 96352985 PubMed ID: 8748272
TITLE: Genetic diversity among Mycobacterium avium complex AccuProbe-positive isolates.
AUTHOR: Soini H; Eerola E; Viljanen M K
CORPORATE SOURCE: Department in Turku, National Public Health Institute, Turku, Finland.
SOURCE: JOURNAL OF CLINICAL MICROBIOLOGY, **(1996 Jan)** 34 (1) 55-7.
Journal code: HSH; 7505564. ISSN: 0095-1137.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-Z50757; GENBANK-Z50758; GENBANK-Z50759; GENBANK-Z50760; GENBANK-Z50761; GENBANK-Z50762; GENBANK-Z50763; GENBANK-Z50764; GENBANK-Z50765; GENBANK-Z50766; GENBANK-Z50767; GENBANK-Z50768; GENBANK-Z50769; GENBANK-Z50770; GENBANK-Z50771; GENBANK-Z50772; GENBANK-Z50773; GENBANK-Z50774; GENBANK-Z50775; GENBANK-Z50776
ENTRY MONTH: 199610
ENTRY DATE: Entered STN: 19961025
Last Updated on STN: 19980206
Entered Medline: 19961011

AB The partial **32-kDa**-protein gene sequences of 22 Mycobacterium avium complex (MAC) clinical isolates that were positive by the AccuProbe MAC probe only (not by the M. avium or M. intracellulare probe) were determined. The obtained nucleotide sequences were compared with the published sequences for M. **tuberculosis**, M. avium, and M. intracellulare by a sequence analysis program. There was a wide range

of genetic diversity among the strains studied. Most of them (16 of 22) had sequences similar but not identical to those of M. avium and M. intracellulare. These strains were considered to be true MAC strains. Five strains had sequences in the category of the novel MAIX sequence, which was very different from the sequences of other mycobacteria analyzed thus far. In addition to these strains, one isolate had a sequence that differed greatly from the reference sequences. These results support previous findings showing that the MAC probably contains several additional species. Our results also suggest that the MAC AccuProbe may react with strains that do not belong to the MAC.

L11 ANSWER 9 OF 27 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 95336598 MEDLINE
DOCUMENT NUMBER: 95336598 PubMed ID: 7542003
TITLE: Antibody reactivity to mycobacterial 65 kDa heat shock protein: relevance to autoimmunity.
AUTHOR: Karopoulos C; Rowley M J; Handley C J; Strugnell R A
CORPORATE SOURCE: Department of Biochemistry, Monash University, Clayton, Victoria, Australia.
SOURCE: JOURNAL OF AUTOIMMUNITY, **(1995 Apr)** 8 (2) 235-48. Journal code: ADL; 8812164. ISSN: 0896-8411.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199508
ENTRY DATE: Entered STN: 19950905
Last Updated on STN: 19960129
Entered Medline: 19950818

AB Reactivity to the mycobacterial 65 kDa heat shock protein (HSP 65) has been implicated in the pathogenesis of adjuvant arthritis in the rat, and may be involved in the pathogenesis of rheumatoid arthritis or other autoimmune diseases in humans. Accordingly this study sought quantitative or qualitative differences in the antibody reactivity to HSP 65 between normal controls, patients with the multisystem autoimmune diseases, rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) and patients with the mycobacterial infections, **tuberculosis** (TB) and leprosy. Levels of antibodies to recombinant HSP 65 in serum were measured by ELISA in normal subjects and in patients with RA, SLE, TB or leprosy. Antibody reactivity was examined by Western blotting using polypeptide fragments of HSP 65 derived by recombinant DNA techniques, or by digestion with trypsin or cyanogen bromide (CNBr). Reactivity to a synthetic peptide, the adjuvant arthritis T-cell epitope of HSP 65 (180-188), was tested by ELISA. High levels of antibodies to full length recombinant HSP 65 from Mycobacterium bovis were present in all the groups tested. By Western blot analysis, most reactivity with intact HSP 65 was retained in a **32 kDa** tryptic fragment, judged by sequencing and size estimations to represent amino acid residues 118-approximately 388. This sequence included a major T-cell epitope for adjuvant arthritis (180-188), but these nine amino acids were not essential for B-cell reactivity since most sera also reacted with residues 188-540 which lack the T-cell epitope. Moreover, the 180-188 synthetic peptide was unreactive by ELISA, and did not inhibit reactivity with the intact recombinant HSP 65. In conclusion, most individuals had antibodies to mycobacterial HSP 65, presumably resulting from previous bacterial infections. The magnitude of the response was unrelated to the occurrence of systemic autoimmune disease, and the pattern of antibody reactivity

with recombinant and proteolytic fragments of HSP 65 suggests that the major B-cell epitope is conformational and consists of discontinuous regions of the molecule.

L11 ANSWER 10 OF 27 LIFESCI COPYRIGHT 2001 CSA
ACCESSION NUMBER: 95:93357 LIFESCI
TITLE: Antibody reactivity to mycobacterial 65 kDa heat shock protein: Relevance to autoimmunity
AUTHOR: Karopoulos, C.; Rowley, M.J.; Handley, C.J.; Strugnell, R.A.
CORPORATE SOURCE: Cent. Mol. Biol. and Med., Monash Univ., Clayton, Vic. 3168, Australia
SOURCE: J. AUTOIMMUN., (**1995**) vol. 9, no. 2, pp. 235-248. ISSN: 0896-8411.
DOCUMENT TYPE: Journal
FILE SEGMENT: F
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Reactivity to the mycobacterial 65 kDa heat shock protein (HSP 65) has been implicated in the pathogenesis of adjuvant arthritis in the rat, and may be involved in the pathogenesis of rheumatoid arthritis or other autoimmune diseases in humans. Accordingly this study sought quantitative or qualitative differences in the antibody reactivity to HSP 65 between normal controls, patients with the multisystem autoimmune diseases, rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) and patients with the mycobacterial infections, **tuberculosis** (TB) and leprosy. Levels of antibodies to recombinant HSP 65 in serum were measured by ELISA in normal subjects and in patients with RA, SLE, TB or leprosy. Antibody reactivity was examined by Western blotting using polypeptide fragments of HSP 65 derived by recombinant DNA techniques, or by digestion with trypsin or cyanogen bromide (CNBr). Reactivity to a synthetic peptide, the adjuvant arthritis T-cell epitope of HSP 65 (180-188), was tested by ELISA. High levels of antibodies to full length recombinant HSP 65 from Mycobacterium bovis were present in all the groups tested. By Western blot analysis, most reactivity with intact HSP 65 was retained in a **32 kDa** tryptic fragment, judged by sequencing and size estimations to represent amino acid residues 118-similar to 388. This sequence included a major T-cell epitope for adjuvant arthritis (180-188), but these nine amino acids were not essential for B-cell reactivity since most sera also reacted with residues 188-540 which lack the T-cell epitope. Moreover, the 180-188 synthetic peptide was unreactive by ELISA, and did not inhibit reactivity with the intact recombinant HSP 65. In conclusion, most individuals had antibodies to mycobacterial HSP 65, presumably resulting from previous bacterial infections. The magnitude of the response was unrelated to the occurrence of systemic autoimmune disease, and the pattern of antibody reactivity with recombinant and proteolytic fragments of HSP 65 suggests that the major B-cell epitope is conformational and consists of discontinuous regions of the molecule.

L11 ANSWER 11 OF 27 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 95189921 MEDLINE
DOCUMENT NUMBER: 95189921 PubMed ID: 7883881
TITLE: Identification of mycobacteria by PCR-based sequence determination of the 32-kilodalton protein gene.
AUTHOR: Soini H; Bottger E C; Viljanen M K
CORPORATE SOURCE: National Public Health Institute, Department in Turku,

Finland.
SOURCE: JOURNAL OF CLINICAL MICROBIOLOGY, **(1994 Dec)** 32 (12) 2944-7.
Journal code: HSH; 7505564. ISSN: 0095-1137.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-Z33653; GENBANK-Z33654; GENBANK-Z33655; GENBANK-Z33656; GENBANK-Z33657; GENBANK-Z33658; GENBANK-Z33659; GENBANK-Z33660; GENBANK-Z33661; GENBANK-Z33662; GENBANK-Z33663; GENBANK-Z33664; GENBANK-Z33665; GENBANK-Z33666; GENBANK-Z33667
ENTRY MONTH: 199504
ENTRY DATE: Entered STN: 19950425
Last Updated on STN: 19950425
Entered Medline: 19950413

AB In this study, a part of the nucleotide sequence of the mycobacterial **32-kDa** protein gene was determined by PCR-based sequencing. A total of 24 mycobacterial strains, representing 10 species, were studied. Sequences of all tested members of the Mycobacterium **tuberculosis** complex were identical to each other and to the previously published sequence of M. **tuberculosis** H37Rv. The sequences of M. avium and M. intracellulare were different from each other. MAIX strains, identified with the Gen-Probe MAIX test, had sequences identical to each other but clearly different from those of M. avium and M. intracellulare. Each of the other mycobacterial species investigated, i.e., M. kansasii, M. gastri, M. gordonae, and M. malmoense, had a unique species-specific sequence. These results demonstrate that there is variation in the nucleotide sequence of the **32-kDa** protein gene among different mycobacterial species. Thus, we propose that this gene can be used for PCR-based identification of mycobacteria.

L11 ANSWER 12 OF 27 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 94332453 MEDLINE
DOCUMENT NUMBER: 94332453 PubMed ID: 8055192
TITLE: Isolation of a **32 kDa** Mycobacterium **tuberculosis** protein by lectin affinity chromatography.
AUTHOR: Montano L F; Masso F; Paez A; Sandoval S; Vazquez L; Sanchez L; Fournet B; Zenteno E
CORPORATE SOURCE: Depto Biologia Celular, Instituto Nacional de Cardiologia, Tlalpan, Mexico.
SOURCE: COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY. BIOCHEMISTRY AND MOLECULAR BIOLOGY, **(1994 Jun)** 108 (2) 265-72.
Journal code: B1P; 9430606.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199409
ENTRY DATE: Entered STN: 19940920
Last Updated on STN: 19940920
Entered Medline: 19940913

AB A **32 kDa** antigen from delipidated M. **tuberculosis** H37Rv culture filtrate protein extract (CFPE) was purified by affinity chromatography on immobilized Lens culinaris lectin and electroelution. This antigen represents 0.4% of the total CFPE

carbohydrate content and possesses galactose, xylose, mannose and GlcNAc (5:2:3:1 mol. ratio). A monoclonal antibody against the purified antigen reacted with the **32 kDa** as well as a 30 kDa antigen in H37Rv CFPE, thus suggesting that both antigens represent closely related allelomorphic forms of the same antigen.

L11 ANSWER 13 OF 27 BIOTECHDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1995-04537 BIOTECHDS
TITLE: Production of antigen 85 complex proteins from Bacillus Calmette Guerin (BCG) vaccine, Tice substrain, in a repeated fed-batch reactor;
Mycobacterium leprae, Mycobacterium bovis, Mycobacterium tuberculosis and BCG antigen-85 complex production; application in vaccine production (conference abstract)
AUTHOR: Tian X X; Klegerman M; Groves M J
CORPORATE SOURCE: Univ.Illinois-Inst.Tuberc.Res.
LOCATION: Institute for Tuberculosis Research, University of Illinois at Chicago (M/C 964), 840 W. Taylor (2014 SEL), Chicago, IL 60607, USA.
SOURCE: Pharm.Res.; (**1994**) 11, 10, Suppl., S93
CODEN: PHREEB
ISSN: 0724-8741
AAPS 94, San Diego, California, 6-10 November, 1994.
DOCUMENT TYPE: Journal
LANGUAGE: English
AN 1995-04537 BIOTECHDS
AB The antigen 85 complex is a group of proteins, 30-**32** **kDa**, identified as being the main antigens produced by Mycobacterium **tuberculosis** and therefore constitute collectively an important component of any **tuberculosis** vaccine. This antigenic group is also associated with the cell walls of Mycobacterium leprae and Mycobacterium bovis and is readily obtained from spent culture fluids of the commercial attenuated M. bovis, Bacillus Calmette Guerin, BCG, vaccine. The vaccine is usually produced by surface culture. Factors involved in the growth of the Tice substrain in repeat fed-batch fermentors. Using a gravity glass separation column attached to a New Brunswick 5.0 l BioFlo III fermentor enabled samples of the growth medium to be taken and total protein determined by a non-specific procedure (BCA). Ag 85 proteins were identified with SDS-PAGE and quantitated using the monoclonal antibody HYT-27 in an ELISA assay procedure. Although protein levels steadily increased, the Ag 85 peaked after 13 days growth, thereafter declining. The results from surface cultures of the same organism tended to parallel those obtained by deep fermentation. (0 ref)

L11 ANSWER 14 OF 27 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1993:433104 BIOSIS
DOCUMENT NUMBER: PREV199396087729
TITLE: Purification of 30-kDa and **32-kDa** protein antigens from Mycobacterium **tuberculosis** and activation of human monocytes by lymphokines.
AUTHOR(S): Choi, Tae-Kyung; Kim, Hwa-Jung; Jo, Eun-Gyeong; Park, Jeong-Kyu; Paik, Tae-Hyun
CORPORATE SOURCE: Dep. Microbiol., Coll. Med., Chungnam Natl. Univ., Tae Jeon
North Korea

SOURCE: Journal of the Korean Society for Microbiology, (1993) Vol. 28, No. 2, pp. 113-130.
ISSN: 0253-3162.
DOCUMENT TYPE: Article
LANGUAGE: Korean
SUMMARY LANGUAGE: Korean; English
AB Identification and characterization of individual components of M. **tuberculosis** have long been a focus of research on **tuberculosis**. The 30-kDa antigens are major constituents of M. bovis BCG and M. **tuberculosis** culture fluids. Because 30-kDa and **32-kDa** antigens are partially identical, these antigens are difficult to purify in large amounts by biochemical techniques. This study was performed to purify the 30-kDa and **32-kDa** antigens to homogenity from the unheated culture filtrate of M. **tuberculosis** H-37Rv. The 30-kDa and **32-kDa** antigen complexes were primarily purified by 50% ammonium sulfate precipitation, hydroxylapatite chromatography and Sephadex G-75 gel filtration. And then further purification for separation of the two antigens was accomplished on preparative isoelectric focusing (IEF). Recovery of 30-kDa and **32-kDa** antigens during above the purification procedures were 28% and 14%, respectively, and 147.0 and 59.8-fold purification were showed, respectively. On silver stained SDS-PAGE gels, the purified **32-kDa** antigen gave a single band at **32-kDa** molecule, while 30-kDa antigen gave one major band at 30-kDa molecule and faint additional bands at **32-kDa**. The pI of 30- and **32-kDa** antigens were 4.3 and 4.6, respectively. The partial identity between these two antigens was observed through the same pattern of reactivity of antigens in the ELISA and imunodiffusion. To the immunological activity of the purified 30-kDa antigen, we also examined the potential of lymphokines from 30-kDa antigen stimulated mononuclear cells to enhance H-2O-2 and O-2- releasing capacity of human monocyte-derived macrophage (MDM). The lymphokines were produced by stimulation of unseparated mononuclear cells or non-adherent mononucler cells of PPD (+) or PPD (-) persons with 30-kDa antigen. Monocytes were matured into MDM by culture for 4 days and then were activated by the lymphokines. LK prepared from PPD (+) person enhanced H-2O-2 and O-2- production by MDM in dose- and time-dependent fashion. Esecially, MDM incubated with a high dilution (1/64) of PPD (+) Ag-LK demonstrated an enhanced capacity to release H-2O-2 and O-2-. However, treatment of MDM with control-LK or PPD (-)-LK do not lead to increase in the H-2O-2 and O-2- production. Considerable variation of H-2O-2 and O-2- releasing capacity of MDM from 9 healthy subjects were observed. MDM activated by rIFN-gamma showed the highest production and MDM treated by PPD (+) Ag-LK released more H-2O-2 and O-2- than that treated by PPD (+) MAg-LK. H-2O-2 and O-2- releasing capacity of MDM of 5 **tuberculosis** patients were same or higher than that of healthy subjects, and PPD (-)LK treated MDM also enhanced H-2O-2 and O-2- production. These results suggest that the 30- and **32-kDa** antigens could be effectively purified by the IEF and reactions of partial identity between the two antigens were found. However, 30-kDa antigen was the more immunogenic antigen than **32-kDa**. The 30-kDa antigen may be important in the cell mediated response to infection with M. **tuberculosis**, and MDM from **tuberculosis** patients was able to generate H-2O-2 and O-2- by lymphokines.

L11 ANSWER 15 OF 27 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 93014155 MEDLINE
DOCUMENT NUMBER: 93014155 PubMed ID: 1398959
TITLE: Mycobacterium leprae produces extracellular homologs of the antigen 85 complex.
AUTHOR: Pessolani M C; Brennan P J
CORPORATE SOURCE: Department of Microbiology, Colorado State University, Fort Collins 80523.
CONTRACT NUMBER: AI 05074 (NIAID)
SOURCE: INFECTION AND IMMUNITY, **(1992 Nov)** 60 (11) 4452-9.
Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199211
ENTRY DATE: Entered STN: 19930122
Last Updated on STN: 19950206
Entered Medline: 19921125

AB The antigen 85 complex is a set of at least three closely related secreted proteins (85A, 85B, and 85C) of 30 to **32 kDa** produced by Mycobacterium **tuberculosis** and other mycobacteria. Their prominence in Mycobacterium leprae, the one obligate intracellular pathogen of the genus, had been assumed on the basis of immunological evidence and proof of the existence of the gene encoding the 85B protein of the complex. We have now observed the production of this family of proteins by M. leprae through analysis of various fractions by Western blotting (immunoblotting) with monospecific rabbit antisera raised against the individual Mycobacterium bovis BCG 85A, 85B, and 85C proteins. A predominant cross-reactive band with an apparent molecular mass of 30 kDa was detected in extracts of nondisrupted whole M. leprae and in soluble fractions prepared from the tissues of M. leprae-infected armadillos. Further studies of the subcellular distribution of this protein within the bacterium confirmed that it is secreted by the organism, an observation that explains past difficulties in detecting the antigen 85 complex in M. leprae. Confirmation that the M. leprae product is a member of the antigen 85 complex was obtained by comparison of peptide fingerprints with those from the BCG product. The pattern of reactivity of the M. leprae antigen 85 complex with anti-M. bovis BCG 85B serum, as well as two-dimensional electrophoresis, established that the 85B component was the predominant member of the complex in M. leprae. The fibronectin-binding capacity of the M. leprae and BCG 85 complexes was reinvestigated by new approaches and is questioned. Nevertheless, the results obtained with the native proteins reinforce previous reports, derived primarily from the use of homologous proteins, that the antigen 85 complex is one of the dominant protein immunogens of the leprosy bacillus.

L11 ANSWER 16 OF 27 MEDLINE DUPLICATE 11
ACCESSION NUMBER: 92364035 MEDLINE
DOCUMENT NUMBER: 92364035 PubMed ID: 1500509
TITLE: Detection and identification of mycobacteria by amplification of a segment of the gene coding for the 32-kilodalton protein.

AUTHOR: Soini H; Skurnik M; Liippo K; Tala E; Viljanen M K
CORPORATE SOURCE: Department of Turku, National Public Health Institute, Finland.
SOURCE: JOURNAL OF CLINICAL MICROBIOLOGY, **(1992 Aug)** 30 (8) 2025-8.
Journal code: HSH; 7505564. ISSN: 0095-1137.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199209
ENTRY DATE: Entered STN: 19920925
Last Updated on STN: 19920925
Entered Medline: 19920911

AB A polymerase chain reaction (PCR) assay for the rapid detection of mycobacterial DNA is described. Oligonucleotide primers, derived from the sequence of a gene coding for the **32-kDa** antigen of Mycobacterium **tuberculosis**, amplified DNA from all 28 species of mycobacteria tested. All nonmycobacterial species tested were negative. An oligonucleotide probe hybridized to the PCR products of the strains belonging to the M. **tuberculosis** complex. This method could detect as little as 50 fg, as tested with purified M. **tuberculosis** DNA. By this amplification method, 127 sputum specimens were tested, with 7.9% of the specimens proving to be inhibitory in PCR. The sensitivity of detection by PCR compared with that by culture was 55.9%; when the inhibitory specimens were excluded, the sensitivity was 70.4%. The specificity of PCR combined with hybridization was 100%.

L11 ANSWER 17 OF 27 LIFESCI COPYRIGHT 2001 CSA
ACCESSION NUMBER: 91:82559 LIFESCI
TITLE: Nucleotide sequence of the gene coding for the 85-B antigen of Mycobacterium leprae .
AUTHOR: de Mendonca Lima, L.; Content, J.; van Heuverswyn, H.; Degrave, W.
CORPORATE SOURCE: Dep. Biochem. and Mol. Biol. and Lab. Hanseniasis, Dep. Trop. Med., Oswaldo Cruz Inst., Ave. Brasil 4365, Rio de Janeiro, RJ, CEP 21045, Brazil
SOURCE: NUCLEIC ACIDS RES., (**1991**) vol. 19, no. 20, p. 5789.
DOCUMENT TYPE: Journal
FILE SEGMENT: J; N; G; L
LANGUAGE: English

AB Antigens of the 85 complex, present in culture supernatants of a variety of mycobacteria, play an important role in humoral and cellular immune responses. As no data on the structure of the corresponding M. leprae proteins were available, we decided to clone the genes coding for the different antigens belonging to this complex. Using a Eag I/BstE II 900 bp DNA fragment coding for part of the M. **tuberculosis** **32 kDa** protein (antigen 85-A; 2) we screened a M. leprae genomic library constructed in the vector lambda-dash. Several clones were isolated and were shown, through hybridization and sequence analysis, to code for antigens 85-A (**32 kDa**), 85-C (33 kDa) and 85-B (28 kDa, alpha antigen), as judged from sequence homology with the corresponding M. bovis BCG, M. **tuberculosis** and M. kansasii genes. Here we show that the presumably secreted M. leprae 85-B antigen shares 86.3% and 84.5% homology, respectively, to the M. kansasii and M. bovis BCG corresponding mature antigens. A presumable 38 amino acid

signal peptide, as judged by sequence homology and hydrophobicity, precedes the 289 amino acid long mature protein. In the 5' upstream sequence, putative promoter and ribosome binding site sequences could be identified which are homologous to the E. coli consensus.

L11 ANSWER 18 OF 27 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 91348869 MEDLINE
DOCUMENT NUMBER: 91348869 PubMed ID: 1715324
TITLE: The genes coding for the antigen 85 complexes of Mycobacterium tuberculosis and Mycobacterium bovis BCG are members of a gene family: cloning, sequence determination, and genomic organization of the gene coding for antigen 85-C of M. tuberculosis.
AUTHOR: Content J; de la Cuvellerie A; De Wit L; Vincent-Levy-Frebault V; Ooms J; De Bruyn J
CORPORATE SOURCE: Department of Virology, Institut Pasteur du Brabant, Brussels, Belgium.
SOURCE: INFECTION AND IMMUNITY, **(1991 Sep)** 59 (9) 3205-12.
Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-S54032; GENBANK-S70201; GENBANK-S70204; GENBANK-X06392; GENBANK-X14442; GENBANK-X16435; GENBANK-X52899; GENBANK-X53033; GENBANK-X57105; GENBANK-X57229; GENBANK-X59240
ENTRY MONTH: 199110
ENTRY DATE: Entered STN: 19911020
Last Updated on STN: 19960129
Entered Medline: 19911002

AB A gene encoding the 33-kDa secreted protein of Mycobacterium **tuberculosis** (antigen 85-C) was isolated and sequenced. The corresponding DNA sequence contains a 1,020-bp coding region. The deduced amino acid sequence corresponds to a 340-residue protein consisting of a 46-amino-acid signal peptide and a 294-amino-acid mature protein. Comparison with previously described genes for the 30-kDa antigen (the alpha antigen of M. bovis BCG, also called antigen 85-B) and the **32-kDa** antigens from M. bovis BCG and M. **tuberculosis** (antigens 85-A) indicates that the three genes share considerable sequence homology (70.8 to 77.5%) but may also code for distinctive epitopes. Strong differences among the three sequences are clearly visible upstream and downstream from the region coding for the mature proteins. The three genes have been detected in the genome of M. bovis BCG by Southern blot hybridization with three type-specific probes. Furthermore, hybridization of large DNA fragments (100 to 1,000 kbp) from M. **tuberculosis** separated by pulsed-field gel electrophoresis showed that the three genes coding for the antigen 85 complex are not clustered within the bacterial genome.

L11 ANSWER 19 OF 27 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 91340353 MEDLINE
DOCUMENT NUMBER: 91340353 PubMed ID: 1714879
TITLE: Preparation of monoclonal antibodies to the beta A subunit of ovarian inhibin using a synthetic peptide immunogen.
AUTHOR: Groome N; Lawrence M
CORPORATE SOURCE: School of Biological and Molecular Sciences, Oxford Polytechnic, Headington, U.K.
SOURCE: HYBRIDOMA, **(1991 Apr)** 10 (2) 309-16.

Journal code: GFS; 8202424. ISSN: 0272-457X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199109
ENTRY DATE: Entered STN: 19911013
Last Updated on STN: 19990129
Entered Medline: 19910920

AB Seven different synthetic peptides were prepared corresponding to regions of the beta A subunit of **32 KDa** human ovarian inhibin predicted to contain possible continuous B cell epitopes. These were coupled to tuberculin as a carrier and used to immunize mice previously given a priming dose of human **tuberculosis** vaccine. Only one of these peptides, corresponding to sequence 82-114, consistently gave good titres of antibodies reactive with intact **32 KDa** bovine inhibin by ELISA. From one of the mice immunized with this peptide, six stable hybridomas were prepared. Five of these secreted an IgM and the sixth (clone E4) secreted an IgG2b antibody. Immunoblotting experiments on follicular fluid concentrates, after treatment with sodium dodecyl sulphate and mercaptoethanol, showed strong reactivity of the antibody from clone E4 only with bands of about 13 kDa and 58 kDa corresponding to forms of the beta A subunit previously described. This monoclonal antibody, and an antibody to the alpha subunit previously made in this laboratory using synthetic peptides, should prove useful reagents for further study of inhibins and activins.

L11 ANSWER 20 OF 27 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1991:345913 BIOSIS
DOCUMENT NUMBER: BA92:45288
TITLE: PURIFICATION AND IMMUNOCHEMICAL CHARACTERIZATION OF ALPHA ANTIGEN FROM THE CULTURE FILTRATE OF MYCOBACTERIUM-TUBERCULOSIS.
AUTHOR(S): KIM S-K; PAIK T-H; PARK H-J; CHOI T-K
CORPORATE SOURCE: DEP. MICROBIOL., CHUNGNAM NATL. UNIV., COLL. MED., TAEJON, KOREA.
SOURCE: J KOREAN SOC MICROBIOL, (1991) 26 (1), 45-60.
CODEN: TMHCDX. ISSN: 0253-3162.
FILE SEGMENT: BA; OLD
LANGUAGE: Korean

AB .alpha.-antigen from the culture filtrate of Mycobacterium **tuberculosis** H37Rv was purified by improved purification procedures and immunoaffinity chromatography using rabbit anti-.alpha. serum. Improved purification procedures were involved 50% ammonium sulfate precipitation, hydroxylapatite chromatography, Sephadex G-75 chromatography and preparative natural polyacrylamide gel electrophoresis (PAGE). We also produced seven monoclonal antibodies against .alpha.-antigen by hybridoma technique and analyzed immunochemical characterizations of .alpha.-antigen by SDS-PAGE, ELISA and Western blot using monoclonal antibodies. The results were summarized as follows: 1. The purification of .alpha.-antigen from the unheated culture filtrate by 50% ammonium sulfate precipitation, hydroxylapatite chromatography and Sephadex G-75 chromatography resulted in recovery of 16% of .alpha.-antigen and 28.5-fold purification. .alpha.-antigen purified further by preparative natural-PAGE showed a single distinctive band of

KDa molecule on SDS-PAGE. 2. The molecular weight of .alpha.-antigen was 30,000 as determined by SDS-PAGE and Sephadex G-75 chromatography. 3. .alpha.-antigen was purified by immunoaffinity chromatography as single step. The affinity purified .alpha.-antigen showed a band of 30 KDa molecule and showed another contaminated band of **32 KDa** molecule on SDS-PAGE. 4. By Western blot analysis, seven monoclonal antibodies against .alpha.-antigen showed a distinct band of 30 KDa molecule and multiple different bands between 25 KDa and 65 KDa molecules.

5. All monoclonal antibodies produced were revealed strong cross reactivities with the sonicate antigens of slow growing and rapid growing mycobacteria by ELISA. These results suggest that .alpha.-antigen, 30 KDa of molecular weight, could be purified well by the improved above purification procedures and contains many cross-reactive antigenic determinants on the molecule.

L11 ANSWER 21 OF 27 LIFESCI COPYRIGHT 2001 CSA
ACCESSION NUMBER: 90:34651 LIFESCI
TITLE: Nucleotide sequence of the 32 kDa-protein gene (antigen 85 A) of Mycobacterium bovis BCG.
AUTHOR: De Wit, L.; de la Cuvellerie, A.; Ooms, J.; Content, J.
CORPORATE SOURCE: Inst. Pasteur Brabant, Dep. Virol., B-1180 Brussels, Belgium
SOURCE: NUCLEIC ACIDS RES., (**1990**) vol. 18, no. 13, p. 3995.
DOCUMENT TYPE: Journal
FILE SEGMENT: J; N; G; L
LANGUAGE: English
AB We have isolated and sequenced a BCG genomic clone. The latter contains a 1299 base pair sequence corresponding to the **32 kDa** -protein gene which is identical to that from M. **tuberculosis** except for a silent single nucleotide change at position 1023. It contains a 1014 base pair coding region. The deduced amino acid sequence corresponds to a 338-residue protein including a 43-residue-long putative signal peptide required for the secretion of the 295-amino-acid-long mature protein.

L11 ANSWER 22 OF 27 MEDLINE
ACCESSION NUMBER: 91075161 MEDLINE
DOCUMENT NUMBER: 91075161 PubMed ID: 2123996
TITLE: The **32 kDa** protein antigen of M. bovis B.C.G. and M. **tuberculosis** H37Rv.
AUTHOR: De Bruyn J; Huygen K; Van Vooren J P; Content J; Turneer M
CORPORATE SOURCE: Pasteur Institute of Brabant, Brussels, Belgium.
SOURCE: TROPICAL MEDICINE AND PARASITOLOGY, **(1990 Sep)** 41 (3) 331-2. Ref: 13
Journal code: TRP; 8503728. ISSN: 0177-2392.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199101
ENTRY DATE: Entered STN: 19910308
Last Updated on STN: 19910308
Entered Medline: 19910124

L11 ANSWER 23 OF 27 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1991:310399 BIOSIS
DOCUMENT NUMBER: BR41:18989
TITLE: THE **32 KDA** PROTEIN ANTIGEN OF MYCOBACTERIUM-BOVIS BCG AND MYCOBACTERIUM-**TUBERCULOSIS** H37RV.
AUTHOR(S): DE BRUYN J; HUYGEN K; VAN VOOREN J-P; CONTENT J; TURNEER M
CORPORATE SOURCE: INST. PASTEUR BRABANT, RUE ENGELAND 642, B-1180 BRUXELLES, BELGIUM.
SOURCE: INDO-EC SYMPOSIUM ON LEPROSY AND OTHER MYCOBACTERIAL DISEASES, LONAVLA, INDIA, NOVEMBER 6-9, 1989. TROP MED PARASITOL, (1990) 4 (3), 331-332.
CODEN: TMPAEY. ISSN: 0177-2392.
DOCUMENT TYPE: Conference
FILE SEGMENT: BR; OLD
LANGUAGE: English

L11 ANSWER 24 OF 27 MEDLINE DUPLICATE 14
ACCESSION NUMBER: 89379378 MEDLINE
DOCUMENT NUMBER: 89379378 PubMed ID: 2506131
TITLE: Cloning, sequence determination, and expression of a 32-kilodalton-protein gene of Mycobacterium tuberculosis.
AUTHOR: Borremans M; de Wit L; Volckaert G; Ooms J; de Bruyn J; Huygen K; van Vooren J P; Stelandre M; Verhofstadt R; Content J
CORPORATE SOURCE: Pasteur Institute Brabant, Brussels, Belgium.
SOURCE: INFECTION AND IMMUNITY, **(1989 Oct)** 57 (10) 3123-30.
Journal code: GO7; 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198910
ENTRY DATE: Entered STN: 19900309
Last Updated on STN: 19900309
Entered Medline: 19891023

AB We describe the identification of the gene encoding an immunodominant 32-kilodalton (kDa) protein of Mycobacterium **tuberculosis**. The **32-kDa** antigen is abundantly secreted into the culture supernatant of a variety of mycobacteria and appears to be a major stimulant of cellular and humoral immunity against mycobacteria. Recombinant clones expressing a 140- or 125-kDa beta-galactosidase fusion protein reactive with rabbit polyclonal anti-**32 kDa** protein serum were detected. The corresponding DNA sequence contains a 1,008-base-pair coding region. The deduced amino acid sequence corresponds to a 336-residue protein including the previously determined NH2-terminal sequence of the **32-kDa** protein (J. De Bruyn, K. Huygen, R. Bosmans, M. Fauville, R. Lippens, J. P. Van Vooren, P. Falmagne, M. Weckx, H. G. Wiker, M. Harboe, and M. Turneer, Microb. Pathog. 2:351-366, 1987). Upstream of this NH2-terminal region, the gene codes for a signal peptide required for the secretion of a 294-amino-acid-long mature protein. A putative promoter sequence could be located upstream of the open reading frame. Comparison of the M. **tuberculosis 32-kDa** antigen with the Mycobacterium bovis BCG alpha-antigen (K. Matsuo, R. Yamaguchi, A. Yamazaki, H. Tasaka, and T. Yamada, J. Bacteriol. 170:3847-3854, 1988) revealed 73.8% homology between DNA sequences and 72.8% homology between amino acid sequences (signal and mature protein). Finally, the 140-kDa fusion protein could selectively be recognized by human tuberculous sera.

This result confirms our previous finding that the **32-kDa** antigen could be a valuable tool for the serological diagnosis of **tuberculosis**. Moreover, the availability of recombinant proteins opens perspectives for the localization of relevant B- and T-cell epitope regions on the **32-kDa** antigen.

L11 ANSWER 25 OF 27 MEDLINE DUPLICATE 15
ACCESSION NUMBER: 90134620 MEDLINE
DOCUMENT NUMBER: 90134620 PubMed ID: 2515338
TITLE: Production of monoclonal antibodies for Mycobacterium tuberculosis Aoyama-B and immunological activity of the affinity-purified antigens.
AUTHOR: Tsuyuguchi T
SOURCE: KEKKAKU, **(1989 Dec)** 64 (12) 761-75.
Journal code: KUO; 0422132. ISSN: 0022-9776.
PUB. COUNTRY: Japan
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Japanese
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199003
ENTRY DATE: Entered STN: 19900328
Last Updated on STN: 19900328
Entered Medline: 19900314

AB Nine hybridomas of BALB/c mouse, producing monoclonal antibodies (MAb: MTA 1-9) directed to Mycobacterium **tuberculosis** AOYAMA-B (M. tbc AB), were established. The MAbs reacted to M. tbc AB antigens, forming a main band of **32 KD**, 24 KD or 19 KD on immunoblotting, except MTA 1 that reacted with 58 KD in addition to **32 KD** antigen. Immunoglobulin isotypes of MAbs were IgM, IgG 1 or IgG 2b. On a mode of reactivity of ELISA against various mycobacterial antigens, MAbs were divided into two categories: those reacting to human type mycobacterial antigens alone and those reacting both to the human and non-human type. The MAbs could further be classified into 4 groups by subtle discrimination of ELISA reactivity to the antigens. Three kind of MAb-affinity-purified antigens (MAb-Ag) from M. tbc AB were obtained. Immunogenic activities of the three MAb-Ags, together with that of control PPDs were examined. Every MAb-Ag showed positive reaction in ELISA, DTH skin test and [3H]TdR incorporation of lymphnode cells, on M. tbc AB sensitized rabbit, guinea pig and rat, respectively, with almost equal level of those of PPDs. However, in some occasions, MAb-Ags positively reacted in animals primed by non-human type mycobacteria. It was discussed that whether a homogeneous antigen like MAb-Ag, bearing limited number of epitopes, could be as immunogenic as PPDs consisting of multi-components.

L11 ANSWER 26 OF 27 MEDLINE DUPLICATE 16
ACCESSION NUMBER: 88126912 MEDLINE
DOCUMENT NUMBER: 88126912 PubMed ID: 3124263
TITLE: Specific lymphoproliferation, gamma interferon production, and serum immunoglobulin G directed against a purified **32 kDa** mycobacterial protein antigen (P32) in patients with active **tuberculosis**.
AUTHOR: Huygen K; Van Vooren J P; Turneer M; Bosmans R; Dierckx P; De Bruyn J
CORPORATE SOURCE: Instituut Pasteur van Brabant, Brussels, Belgium.
SOURCE: SCANDINAVIAN JOURNAL OF IMMUNOLOGY, **(1988 Feb)** 27

(2) 187-94.
Journal code: UCW; 0323767. ISSN: 0300-9475.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198803
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19900308
Entered Medline: 19880318

AB Twenty-one patients treated for active **tuberculosis** were examined for immune reactivity to purified protein derivative (PPD) and to a purified **32-kDa** protein antigen (P32) from Mycobacterium bovis, strain BCG. Lymphoproliferation of peripheral blood leucocytes to PPD and P32 was positive in 95% and 71% of the patients respectively. A positive IFN-gamma response was detected in 62% against PPD and in 48% against P32. Low blastogenesis and IFN-gamma production were observed, especially in patients with poor general health and advanced tuberculous lesions. Twelve out of twelve (100%) of the tuberculin-positive healthy volunteers responded to PPD and P32 with mean lymphoproliferation and IFN-gamma values that were higher than in the patient group. Twelve tuberculin-negative control subjects were completely unreactive to PPD and P32 antigen. On the other hand, IgG antibodies in the serum were detected in 95% of the patients against PPD, in 77% of the patients against P32 but in none of the tuberculin-positive or negative healthy volunteers. The highest IgG levels against PPD were found in those patients with the lowest in vitro lymphoproliferation and IFN-gamma production (r = -0.54; P less than 0.05). Nonspecific interferon production following induction with Newcastle disease virus, Corynebacterium parvum, or phytohaemagglutinin was comparable in the control and patient groups. Finally, low IFN-alpha titres were detected in the serum of about 50% of the patients.

L11 ANSWER 27 OF 27 MEDLINE DUPLICATE 17
ACCESSION NUMBER: 86291713 MEDLINE
DOCUMENT NUMBER: 86291713 PubMed ID: 2426875
TITLE: The patterns of complex and partially purified mycobacterial antigens in macrophage migration inhibition testing.
AUTHOR: Kubin M; Wisingerova E; Pekarek J; Prochazka B
SOURCE: ZENTRALBLATT FUR BAKTERIOLOGIE, MIKROBIOLOGIE, UND HYGIENE.
SERIES A, MEDICAL MICROBIOLOGY, INFECTIOUS DISEASES, VIROLOGY, PARASITOLOGY, **(1986 May)** 261 (3) 362-9.
Journal code: Y55; 8403032. ISSN: 0176-6724.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198609
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 19990129
Entered Medline: 19860918

AB In rabbits immunized intratarsally by M. **tuberculosis**, M. kansasii and M. avium the responses to homologous and heterologous antigens were assessed by direct and indirect macrophage migration

inhibition tests. Complex cytoplasmic antigens were obtained by disruption of bacterial mass and by ultracentrifugation of the supernatants. The partially purified antigens were prepared by gel chromatography of the complex antigens on a Sephadex G 150 column. The middle fraction (260/280 ratio approx. 1, molecular weight approx. **32 KD**) was employed as partially purified antigen. In the direct tests the migration activity of immune spleen macrophages was significantly reduced by homologous complex and partially purified antigens (MI = 0.63 to 0.72) and it differed significantly from responses obtained with heterologous antigens (MI = 0.75 to 0.92); however, these were still lower than those in nonimmunized control animals where MI ranged from 0.89 to 1.01. In the indirect tests, the strongest responses were recorded again with homologous complex and partially purified antigens (MI = 0.43 to 0.53). The responses in heterologous systems differed even more markedly than in direct tests (MI = 0.65 to 0.81); and, these were again still significantly lower than in control animals (MI = 0.89 to 0.98). In both direct and indirect tests, the complex and partially purified antigens did not vary substantially in their immunogenic capacity. The presence of cross-reacting responses in heterologous systems can be explained by a close relatedness of mycobacteria used in the immunization schedule and by the presence of common epitopes in complex and purified testing antigens.

L11 ANSWER 21 OF 27 LIFESCI COPYRIGHT 2001 CSA
ACCESSION NUMBER: 90:34651 LIFESCI
TITLE: Nucleotide sequence of the 32 kDa-protein gene (antigen 85 A) of Mycobacterium bovis BCG.
AUTHOR: De Wit, L.; de la Cuvellerie, A.; Ooms, J.; Content, J.
CORPORATE SOURCE: Inst. Pasteur Brabant, Dep. Virol., B-1180 Brussels, Belgium
SOURCE: NUCLEIC ACIDS RES., (**1990**) vol. 18, no. 13, p. 3995.
DOCUMENT TYPE: Journal
FILE SEGMENT: J; N; G; L
LANGUAGE: English

AB We have isolated and sequenced a BCG genomic clone. The latter contains a 1299 base pair sequence corresponding to the **32 kDa** -protein gene which is identical to that from M. **tuberculosis** except for a silent single nucleotide change at position 1023. It contains a 1014 base pair coding region. The deduced amino acid sequence corresponds to a 338-residue protein including a 43-residue-long putative signal peptide required for the secretion of the 295-amino-acid-long mature protein.

L2 ANSWER 1 OF 1 MEDLINE

ACCESSION NUMBER: 91186827 MEDLINE

DOCUMENT NUMBER: 91186827 PubMed ID: 2082148

TITLE: Isolation and characterization of efficient plasmid transformation mutants of Mycobacterium smegmatis.

AUTHOR: **Snapper S B**; Melton R E; Mustafa S; Kieser T; Jacobs W R Jr

CORPORATE SOURCE: Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York 10461.

CONTRACT NUMBER: 5T32GM7288-15 (NIGMS)
AI23545 (NIAID)
AI26170 (NIAID)

SOURCE: MOLECULAR MICROBIOLOGY, **(1990 Nov) 4** (11) **1911-9**.
Journal code: MOM; 8712028. ISSN: 0950-382X.

PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199105

ENTRY DATE: Entered STN: 19910526
Last Updated on STN: 19910526
Entered Medline: 19910503

AB Recent development of vectors and methodologies to introduce recombinant DNA into members of the genus Mycobacterium has provided new approaches for investigating these important bacteria. While most pathogenic mycobacteria are slow-growing, Mycobacterium smegmatis is a fast-growing, non-pathogenic species that has been used for many years as a host for mycobacteriophage propagation and, recently, as a host for the introduction of recombinant DNA. Its use as a cloning host for the analysis of mycobacterial genes has been limited by its inability to be efficiently transformed with plasmid vectors. This work describes the isolation and characterization of mutants of M. smegmatis that can be transformed, using electroporation, at efficiencies 10(4) to 10(5) times greater than those of the parent strain, yielding more than 10(5) transformants per microgram of plasmid DNA. The mutations conferring this efficient plasmid transformation (Ept) phenotype do not affect phage transfection or the integration of DNA into the M. smegmatis chromosome, but seem to be specific for plasmid transformation. Such Ept mutants have been used to characterize plasmid DNA sequences essential for replication of the Mycobacterium fortuitum plasmid pAL5000 in mycobacteria by permitting the transformation of a library of hybrid plasmid constructs. Efficient plasmid transformation of M. smegmatis will facilitate the analysis of mycobacterial gene function, expression and replication and thus aid in the development of BCG as a multivalent recombinant vaccine vector and in the genetic analysis of the virulence determinants of pathogenic mycobacteria.